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The Oxidation of Nicotine. I. Kinetics of the Liquid Phase Reaction Near Room Temperature

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Introduction

Although the biochemical degradation and oxidation of nicotine has been extensively investigated (1), the kinetics of the reaction of oxygen with nicotine has not previously been reported. Obabko (1) reports that heating tobacco containing 25-28% H_2O (in an inert N_2 atmosphere) to 70-75°C. does not deprive the tobacco of its ability to bind O_2 from air under fermentation conditions (35°C. and 75% relative humidity) although oxidase is destroyed. The heat treated tobacco absorbed O_2 intensely initially but decreased with time whereas non-heat treated tobacco reaches a maximum O_2 uptake rate after some time and then decreases. Recently Wada (2) has oxidized nicotine at 30° C. with pure O_2 and found about 20% of the nicotine oxidized in 4 weeks. He found ammonia, methyl amine, nicotinic acid, myosimine, cotinine, nicotyrine and unidentified products.

Experimental

Nicotine was vacuum distilled in a Todd Column packed with glass helixes and the fraction at 97-98° (10 mm.) taken (reflux ratio 10:1). Freshly distilled, water-clear nicotine turns yellow in a few hours when exposed to air but when scaled off in a glass ampule under vacuum it remains colorless for months. Preliminary work was done using an AMINCO Warburg apparatus at 20-40° and with various shaking rates. It was found that with N_2 in the Warburg there was no volume change, with O_2 there was a rapid uptake of gas which was roughly 5 times the uptake rate using air; rates increased with shaking speeds and increased with temperature. Nicotine acidified with HCl, H_2SO_4 and HOAc did not have any O_2 uptake activity indicating that the free base is involved in the oxidation. To obtain kinetic data an apparatus was constructed which consisted of a flat bottom flask of about 50 ml. volume fitted with a ground glass stopper for filling and a side arm which was connected to a 100 ml. gas burette containing sat. NaCl-H_2O and a 3-way stopcock for flushing the apparatus with gas. Oxygen gas at 1 atmosphere pressure was used in all experiments. The reaction flask was mounted on a shaker which had variable amplitude and speed and was immersed in a water bath regulated to $\pm$ 0.1°C. Experiments were performed at various shaking amplitudes and speed with nicotine samples of 1.00-5.00 ml. A sample size of 2.00 ml. and shaking conditions such that rates of oxygen uptake were independent of shaking for the temperature range of 15-40° were used in this study. Rates were found to decrease on aging of freshly distilled nicotine. Vacuum storage prevents aging but air contact during sample removal gradually aged the stored nicotine; this problem was solved by using Matheson Prepurified N_2 (8 ppm O_2) to flush out the nicotine storage flask after each sample removal and in this way identical rates could be obtained from the same nicotine over a period of one week.

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Oxygen was bubbled into nicotine held at temperatures from 50-70°C. The nicotine rapidly turned brown and an infrared band just before 6 microns, absent in pure nicotine, increased in intensity as the experiment continued. The infrared band suggests cotinine is being formed (3). Exodation for several days and then using Frankenburg's (3) technique for isolation of cotinine indicated complex reaction products. Addition of a free radical initiator, α,α'-Azodi-isobutyronitrile (AIBN), in a similar experiment, resulted in more rapid darkening of the nicotine and similar, if not identical, reaction products. Addition of an antioxidant, 4-methyl-2,6-di-tert-butylphenol (BHT), greatly decreased rate of coloration of nicotine on oxidation and inactivated nicotine to O_2 uptake for several hours.

Several experiments were done on a burley tobacco containing 5.1-5.2% nicotine. Small samples of the tobacco were sealed in glass tubes containing N_2, air or O_2 and the tubes heated for various periods of time in a hot air oven. Nicotine was determined by the method of Cundiff (4). No change in nicotine content was found with N_2 at temperatures from 85-125°C. With O_2, the nicotine was reduced to one-half its original value in 300 hours at 85°, 24 hours at 110° and 3 hours at 125°.

Results and Discussion

In Figure 1 typical plots of volume O_2 used vs time are shown. Table 1 tabulates all the results where rates are those from the linear part of the reaction. An Arhenius plot of the maximum rates of table 1 yields an activation energy of 6.81 kcal (least squares). This is a low activation energy compared to the uncatalyzed oxidation of tetralin from 65-130°C., where the overall activation energy is 14.9 $\pm$ 0.6 kcal. (5). If the linear rates are extrapolated back to zero oxygen uptake, and this time called the induction period $t_{ind.}$, a plot of log $(1/t_{ind.})$ vs 1/T would yield an activation energy for the removal of inhibitor. A value of 14.5 kcal. is found. The induction period could be due to formation and decomposition of a nicotine hydroperoxide but it is premature to associate an activation energy with this proposed mechanism. A small evolution of gas in the initial periods of lower temperature rate studies is probably due to decreased gas solubility in the nicotine at lower temperatures.

In the experiments reported here, 2.00 ml. nicotine, at room temp., was used, corresponding to 1.88×10^{-2} moles. For the consumption of one mole O_2 per mole nicotine we would therefore require 421 ml. O_2 at STP. Most experiments were run to a total O_2 consumption of 20-30 ml. (STP) and inhibition (deviation from linear rate) was noted at about 15 ml. (STP) O_2 consumption. The low O_2/nicotine mole ratio at which inhibition is found (about 0.03) together with inhibition by BHT and catalysis by AIBN provides strong evidence of the free-radical nature of the oxidation. This indicates nicotine oxidation follows the general mechanism of olefin oxidations (6) and a nicotine hydroperoxide could be expected as an intermediate. This hydroperoxide would be unstable at room temperature, yielding H_2O_2 (which could form nicotine N-oxide), cotinine, H_2O, and radicals which could form polymers. Further work is underway to isolate the proposed hydroperoxide and provide more details on the mechanism of the reaction.

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Table I

Linear Rates of Oxygen Absorbed by Liquid Nicotine

Run	Temp. °C.	Ml. O_2 (STP) x 10^3 sec x ml. nicotine^{-1}	Induction period sec x 10^{-3}	Remarks
IX-20	15	1.08	5.60	Nicotine stored 24 hrs. under N_2.
XIV-24	20	1.35	2.46	Nicotine stored 10 days under N_2.
V-18	25	1.61	1.60	Nicotine fresh from vacuum dist. No storage.
VII-19	25	1.61	2.12	Nicotine stored 24 hrs. under N_2.
XIII-23	25	1.59	2.22	Nicotine stored 10 days under N_2.
XV-25	25	1.47	1.98	Nicotine stored 14 days under N_2.
XVI-25	25	1.54	1.70	Nicotine stored 14 days under N_2.
XVII-26	25	----	29	Added 50 mg. BHT
XII-22	30	1.73	1.58	Nicotine stored 4 days under N_2.
VIII-20	35	2.09	0.86	Nicotine stored 24 hrs. under N_2.
X-21	35	2.10	0.88	Nicotine stored 3 days under N_2.
XI-21	40	2.34	0.64	Nicotine stored 4 days under N_2.
II-16	25	1.21	2.10	Nicotine yellow from storage one month in poor vacuum.
III-16	25	0.80	----	Reused nicotine from II-16 immediately.
IV-16	25	0.18	----	Reused nicotine from III-16 24 hr. later.

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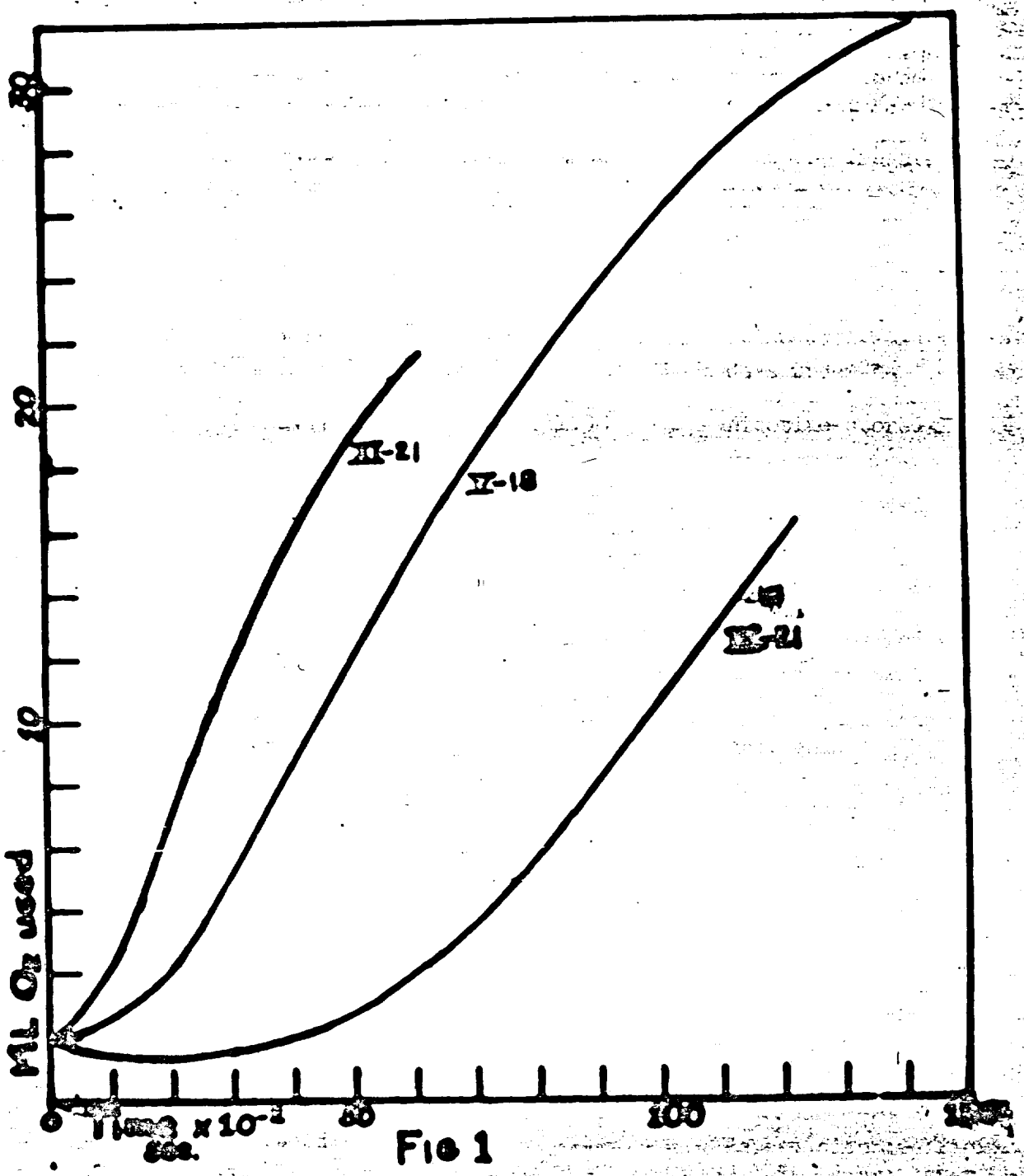

Fig 1

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